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COMPARATIVE HISTOPATHOLOGY
OF ACANTHOCEPHALAN INFECTIONS
IN SOME FRESHWATER FISHES.

11.12.1967 to 1/5/68

10 by
Douglas P. Schelhaas

Bachelor of Arts, Northwestern College, 1967

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A Thesis
Submitted to the Graduate Faculty
of the
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in partial fulfillment of the requirements
for the degree of
Master of Science

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Grand Forks, North Dakota

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1980

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This thesis meets the standards for appearance and conforms to the style and format requirements of the Graduate School of the University of North Dakota, and is hereby approved.

A. William Johnson
Dean of the Graduate School

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ABSTRACT

The objective of this study was to compare the intestinal histopathology of common suckers (Catostomus commersoni), creek chubs (Semotilus atromaculatus) and walleyes (Stizostedion vitreum) infected with acanthocephalans. Special emphasis was placed on the extent and type of host tissue response to these infections.

Intestinal tissues of suckers infected with Octospinifer macilentis and Pomphorhynchus bulbocolli, creek chubs possessing Neoechinorhynchus saginatus and walleyes parasitized by N. cylindratus were excised and fixed in 5% formalin or Bouin's solution. Comparative tissue samples were also taken from uninfected fishes. All tissues were embedded in paraplast and sectioned with a rotary microtome. Sections fixed with Bouin's solution were stained with Mallory's triple connective tissue stain, while those preserved in formalin were stained with Delafield's hematoxylin and eosin Y.

The acanthocephalan infections produced no apparent detriment to the general health of these fishes. However, tissue sections revealed varying amounts of damage and tissue reaction to the parasites. These differences are attributable to the following reasons: 1) depth of attachment of the worm is the primary cause for variations in host tissue reactions; 2) the morphology and depth of mucosal folding determines where and how deeply the worm must attach in order to become firmly anchored; 3) the relative thickness of the lamina propria and tunica muscularis influences the amount of damage resulting from worm attachment.

A comparison of O. macilentis and P. bulbocolli infections in suckers showed that the difference in damage and host response is primarily due to the vast differences between the lengths of their presomas.

INTRODUCTION

General Background

Acanthocephalans, or spiny headed worms, comprise a small phylum of unsegmented, cylindrical, dioecious, endoparasitic worms that lack a digestive tract throughout all stages of their life cycle. The acanthocephalan body is divided into an anterior region, the presoma, and a posterior region, the trunk. Externally, the presoma consists of a spiny proboscis, followed by a non-spinous neck. Internally, the neck continues as the proboscis receptacle into which the proboscis and neck, in most species, is retractable. The proboscis is everted by means of hydrostatic fluids under pressure of body contraction (Meyer and Olsen, 1975), and is retracted by means of the proboscis retractor muscles. The proboscis is the primary holdfast organ by which the adult worm attaches itself to the intestine of its host. It bears on its surface a variable number of sharply pointed, recurved hooks that engage the intestinal tissues of the host. The size, shape, number and arrangement of hooks varies with the taxon. Pathogenesis in the host presumably is based on the depth of penetration of the proboscis and the number of worms present in an individual's intestine. Webster (1943) implied that the presence in the gut of one or two Plagi rhynchus formosus could kill a robin, and Boyd (1951) reported emaciation and blackening of the visceral contents in starlings infected with one to seven worms of this species. Clark, O'Meara and Van Weelden (1958) observed heavy infections of Polymorphous botulus in dead and dying eider ducks. Schmidt, Walley and Wijek (1974) reported prolapsed rectums

and resultant death in the mottled sculpin, Cottus bairdi, due to heavy infections of Acanthocephalus jacksoni. They speculated that the elimination of this fish in a localized region of the stream was due to these infections.

In contrast to the above observation, there are numerous examples of very heavy acanthocephalan infections in fish with a much less definitive pathogenicity (Bullock, 1963). In many cases, fishes with heavy infections appear to suffer, from outward appearances, little if any ill effects. Hine and Kennedy (1974) studied the pathogenicity of Pomphorhynchus laevis in the River Avon, Hampshire England. They examined growth rates and mortality due to acanthocephalan infections in the chub, Leuciscus cephalus, the dace, L. leusiscus, and the grayling, Thymallus thymallus. Infected fish of all three species showed good growth rates when compared with those of these same species in other rivers lacking acanthocephalans. With the exception of two grayling, none of the fish examined appeared to suffer any problems from infections with P. laevis. Hine and Kennedy (op. cit.) stated "it was quite impossible to detect infected fish or to determine the degree of infection by their external appearance."

Since the external manifestations of acanthocephalan infections are relatively rare, one needs to do histopathological examinations to analyze any damage caused by the parasite. This type of study also allows a determination of how the host organism compensates for and protects itself from the parasite. The histopathological literature on acanthocephalan infections in fishes is quite limited. Venard and Warfel (1953) studied infections of Leptorhynchoides thecatus and Neoechinorhynchus

cylindratus in the largemouth bass, Micropterus salmoides, while Esch and Huffines (1973) looked at L. thecatus in the smallmouth bass Micropterus dolomieu. The histopathological manifestations of P. laevis in the chub, dace, and grayling were reported by Hine and Kennedy (1974). Infestations by A. jacksoni in brook trout, Salvelinus fontinalis, in the Atlantic salmon, Salmo salar, the rainbow trout, S. gairdneri, and the brown trout, S. trutta, were documented by Bullock (1963). A study of the histopathology of Echinorhynchus lageniformis infections in the starry flounder, Platichthys stellatus, was reported by Prakash and Adams (1960). Similar studies were conducted by Chaircharn and Bullock (1967) on the common sucker, Catostomus commersoni, infected with Octospinifer macilentis, N. cristus, and P. bulbocolli. They also examined creek chubsuckers, Erimyzon oblongus, infected with N. prolixoides.

Histological changes that occur in fish intestines due to acanthocephalan infections vary with the species of host. Intestinal tissues differ in the nature and thickness of the tunica mucosa, the presence or absence of a stratum compactum and the thickness of the tunica muscularis. The worm's proboscis also influences the response of the host intestine. Length of the proboscis, number of hooks present and variations of the presoma (such as the bulb found in P. bulbocolli) have an effect on the tissue reaction in the host. For example, the depth of penetration of the proboscis plays a major role in the extent of damage to host tissues and on the degree of host response to the worm. In all histopathological studies done on infected fish intestines, there is always mechanical destruction of host tissue. It can range from a simple denudation of the columnar epithelium at the point of attachment of the proboscis, to a perforation of the gut wall. In general, host

reaction consists of a proliferation of fibroblasts in the immediate vicinity of the parasite, thus forming a collagenic capsule. In some instances, eosinophilic and granular cell infiltrations were noted (Prakish and Adams, 1960; Bullock, 1963; Chaircharn and Bullock, 1967; Esch and Huffines, 1973). In other cases, little if any cellular response was noted (Chaircharn and Bullock, 1967; Venard and Warfel, 1953).

The present study was undertaken in order to compare the intestinal histopathology of common suckers, creek chubs and walleyes infected with acanthocephalans. Special emphasis was placed on the degree of host tissue response elicited by the four species of parasites encountered in this study.

Biogeography and Life Histories
of Acanthocephalans

Brief introductory material is herewith presented on the four species of fish-inhabiting acanthocephalans encountered in this study. Three of these (Octospinifer macilentis, Neoechinorhynchus saginatus, and N. cylindratus) are members of the family Neoechinorhynchidae, while the fourth species (Pomphorhynchus bulbocolli) belongs to the family Pomphorhynchidae.

Octospinifer macilentis Van Cleave, 1919

The genus Octospinifer is associated with North American catostomids, with O. macilentis limited to suckers (Catostomus commersoni) in the eastern portions of North America. It has been reported as far west as North Dakota (Voth and Larson, 1968; Sutherland and Holloway, 1979), eastward to Maine (Meyer, 1954), and as far south as Florida (Bangham, 1941). The genus differs from all other Neoechinorhynchidae in possessing eight hooks in each of three circles surrounding a short, globular proboscis (Figure 1). Harms (1965) has shown that eggs from female worms are passed to the environment via the fish's feces. These eggs must be eaten by ostracods in order for larval development to proceed. For O. macilentis no second intermediate host is needed. The infective cystacanth or juvenile larva localizes within the ostracod's hemocoel. Infected ostracods are ingested by suckers while feeding on bottom detritus in lakes and streams. Digestive processes in the fish's intestine release the cystacanth larvae which then attach to the intestinal mucosa. Maturation occurs in approximately 8 to 10 weeks for males and about 16 weeks for females.

Neoechinorhynchus saginatus

Van Cleave and Bangham, 1949

Neoechinorhynchus saginatus is primarily a parasite of the creek chub, Semotilus atromaculatus. This parasite has a geographic range similar to O. macilentis. It is known from North Dakota (Voth and Larson, 1968; Uglem and Larson, 1969), and eastward to Maine (Meyer, 1954). The genus Neoechinorhynchus is typified by a proboscis possessing three circles of hooks with six hooks per circle (Figure 2). The life cycle again includes ostracods with no second intermediate host needed.

Neoechinorhynchus cylindratus

(Van Cleave, 1913) Van Cleave, 1919

Neoechinorhynchus cylindratus (Figure 3) is much less host specific and has a wider geographic range than the two previously mentioned species. It has been reported in fishes from Wisconsin (Anthony, 1963), Florida (Bangham, 1941), Canada (Choquette, 1951), Maine (Meyer, 1954), Texas (Sparks, 1951), South Dakota (Zische and Vaughn, 1962) and Minnesota (Larson, 1966). Van Cleave and Mueller (1934) reported, in studies from Lake Oneida, New York, that the walleye, Stizostedion vitreum, is a preferred definitive host for this worm. Ward (1940) showed that the life cycle of N. cylindratus also utilizes ostracods as intermediate hosts; however, small fishes, such as bluegills can serve as second intermediate hosts. In these fishes, juveniles lodge in the livers until freed in the gut of a larger piscivorous fish, such as the walleye.

Pomphorhynchus bulbocolli

(Linkins, 1919) Van Cleave, 1919

Members of the genus Pomphorhynchus possess long presomas with a

distinctive neck bulb (Figure 4). P. bulbocolli is also very non-specific in terms of its definitive hosts. It has been reported in some 40 genera of freshwater fishes; however, it appears to develop to sexual maturity in only a limited number of species. During 15 years of study, Dr. Bullock reported finding sexually mature P. bulbocolli only in the common sucker, C. commersoni, and the golden shiner, Notemigonus crysoleucas (Chaircharn and Bullock, 1967). Geographically the worm has a transcontinental distribution from British Columbia (Bangham and Adams, 1954) to Maine (DeRoth, 1953). Unlike the previous three species, P. bulbocolli utilizes amphipods as an intermediate host in its life cycle.

MATERIALS AND METHODS

Collection of Materials

Collection of fishes took place in late summer and early autumn of 1979. Common suckers, Catostomus commersoni, infected with Octospinifer macilentis were obtained by seining with a 4½' X 20' minnow seine. The site of collection was the Goose River, Traill County, North Dakota, adjacent to a county road approximately five kilometers south of Hatton, North Dakota. Due to dry weather conditions during the summer of 1979, fish were obtained in abundance by seining intermittent pools of the river. Fish were transported alive in styrofoam coolers to the laboratory. They were then killed by severing the spinal column at the base of the skull. A ventrolateral incision from the operculum to the anus exposed the viscera. The intestine was placed in 0.7% saline solution, opened and examined with the aid of a dissecting microscope. Infected and uninfected portions of the intestine were excised and fixed immediately in 5% formalin or Bouin's solution at room temperature.

Common suckers, infected with Pomphorhynchus bulbocolli, were obtained from the Chanarambie Creek in Pipestone County, Minnesota approximately 0.4 kilometers east of the village of Edgerton, Minnesota. These fish were killed, dissected and examined in the manner previously mentioned. All tissues were fixed in 5% formalin.

Creek chubs, Semotilus atromaculatus, infected with Neoechinorhynchus saginatus, were procured by line fishing, utilizing night crawlers for bait. They were collected from the Rock River in an area approximately 2.5 kilometers northwest of the village of Edgerton, Pipestone County,

Minnesota. These fish were processed similarly to the suckers, but there was considerable detachment of worms from the fixed material. In an attempt to alleviate this problem, through instantaneous death of the worms, infected intestines were opened and fixed in hot Bouin's or 5% formalin. More worms remained attached with hot Bouin's preservation than with hot formalin. None of 14 chubs taken from the Goose River site possessed acanthocephalans. Creek chubs from this site previously were noted to possess N. saginatus (Voth and Larson, 1968; Uglem and Larson, 1969).

Walleye, Stizostedion vitreum, intestines were obtained from fishermen cleaning their catches at Huddle's Resort on the south side of Leech Lake, Minnesota. This site is near the community of Whipholt in Cass County. All walleye materials were fixed in 5% formalin at ambient temperature.

Preparation of Material

Preserved materials were re-examined with a dissecting microscope and both infected and un-infected tissues from each host species were excised for histological preparation. Tissues were dehydrated in an ethanol series, cleared in toluene, and embedded in paraplast (melting point 56-57 degrees centigrade). Sections were cut with a rotary microtome at a thickness of 10 microns and adhered to microscope slides with fresh egg albumen.

Sections preserved in Bouin's fixative were deparaffinized in xylol, rehydrated through a decreasing ethanol series to distilled water, and stained with Mallory's triple connective tissue stain. Best results were obtained by staining for 20 seconds in Mallory I (0.1% acid fuchsin) and

2.5 minutes in Mallory II (0.5% aniline blue, 3% orange G). Mallory's technique was unsuitable for formalin preserved material, but the hematoxylin and eosin stains worked well. This stain also worked well in revealing the distinct cytoplasmic granules of granular cells found in the intestinal tissue. Sections were deparaffinized and hydrated as described above. They were then stained for 2.5 minutes in Delafield's hematoxylin and for 1.5 minutes in eosin Y. All stained sections were dehydrated in ethanol, cleared in xylol, and mounted in Canada balsam.

Identification of Specimens

Identification of acanthocephalan species encountered in this study was based on host specificity and parasite morphology. The latter was ascertained from sectioned material and acetocarmine stained whole mounts. The structure of the presoma was the most important and distinctive criterion in species determinations. Specimens were compared with descriptions and illustrations in the literature.

RESULTS

General Histology of Fish Intestine

For all species of fish examined, normal uninfected and parasite infected tissues were preserved, sectioned, stained and mounted for comparative study. The mucosal layer of all fishes studied consists of simple columnar epithelium and a lamina propria layer (Figure 5). The columnar cells are of two different types. The absorptive cells comprise the bulk of the intestinal epithelium. These have numerous microvilli that project from the free surface into the lumen of the intestine (Jansson and Olsson, 1960). Scattered randomly among the absorptive cells are goblet cells. These unicellular glands secrete mucin which forms mucus when mixed with water in the lumen of the intestine.

Immediately beneath the epithelium is the lamina propria. This is vascularized connective tissue of varying thicknesses. It projects into and forms the supporting core for the villi. The fibrous elements of this layer are mainly collagenous. Within the tunica mucosa of fishes, researchers have reported the presence of other cells, including wandering leukocytes, lymphocytes, fibroblasts and large granular cells (Bolton, 1933; Al-Hussaini, 1949; Bullock, 1963; Chaircharn and Bullock, 1967).

External to the lamina propria a stratum compactum layer may be present, depending upon the species of fish. This is a dense, acellular layer composed of large, wavy, collagenous fibers which encircle the digestive tract. It occurs in the intestine of common suckers, but in some fishes, such as the creek chub, the stratum compactum is replaced

by a submucosa possessing numerous fibroblasts distributed between collagenous fibers. In other fishes, such as the walleye, there is no obvious division of the connective tissue into a lamina propria and submucosa.

The tunica muscularis is composed of a thick inner layer of circular muscle and a thin outer layer of longitudinal muscle. Both of these layers consist of involuntary smooth muscle.

The outer surface of the intestine is covered by a thin connective tissue and mesothelial layer known as the serosa. This is continuous with the abdominal mesenteries.

Specific Histology and Histopathology of Parasitized Intestines

Catostomus commersoni (Lacépède), the Common or White Sucker

Common suckers in this study ranged from 15 to 20 centimeters in total length. Those taken from the Goose River had moderate to heavy infections of Octospinifer macilentis. In spite of this, the fish showed no outward signs of sickness or malnutrition when compared with uninfected fish. In this host, the acanthocephalans always occurred in the posterior one half of the intestine. This region is characterized by low, simple villi sometimes referred to as intestinal folds. Numerous wandering leukocytes at the base of the epithelial cells were noted (Figure 6). This intestine has a distinct stratum compactum and a relatively thin tunica muscularis.

The mechanics of worm attachment involves an initial proboscis penetration of the mucosa, apparently by mechanical agitation and pressure.

To "set the hooks" the proboscis is partially retracted. By this process the trunk is pulled downward into intimate contact with the columnar epithelium of the host intestine. This applies pressure on the hooks and keeps them firmly anchored. Evidence of this force is seen in the elevated gut tissues occurring at attachment sites. Similar mechanics of attachment were noted by Hammond (1967) for Acanthocephalus ranae in toads.

Female worms tend to embed deeper in the gut wall than do male worms. This difference was also noted by Prakish and Adams (1960) for Echinorhynchus lageniformis in flounder. They interpreted this phenomena as an adaptation for greater mobility of the male acanthocephalan to enhance copulation with the more firmly attached female.

Chaircharn and Bullock (1967) reported that the female of Octospinifer macilentis perforates through the mucosa and stratum compactum, but never the tunica muscularis. In my study, the proboscis appears to have gone deeper than the tunica muscularis (Figure 7). This may not contradict Chaircharn and Bullock's (1967) findings for they stated that the tunica muscularis is disrupted at the site of the proboscis and infiltrated with fibrous connective tissue. Initial attachment may not proceed beyond the tunica muscularis. However, it seems probable that as the disruption and fibrosis of the tunica muscularis develops, the worm may slowly penetrate deeper. The appearance would then be that the proboscis had entered or passed through the normal tunica muscularis.

The intestinal wall at the site of attachment also shows a complete destruction of columnar epithelium, lamina propria and stratum compactum. There is also a compression of the epithelium along the trunk of the worm where it extends between the villi (Figure 8). Bullock (1963) felt that such compression is only temporary and varies with the movement of the

intestine. Histochemically he found no difference between compressed and normal cells.

Tissue reaction to the presence of the proboscis of O. macilentis (female) results in the formation of a connective tissue nodule. This is composed of collagenous fibers, fibroblasts and granular cells (Figure 9). In contrast, male worms generally penetrate no deeper than the stratum compactum and a nodule is not usually formed. The stratum compactum beneath the attachment site of males may be normal or somewhat thinner, and there may be some infiltration of the tunica muscularis with fibroblasts and connective tissue (Figure 10).

Common suckers obtained from the Chanarambie Creek were heavily infected with Pomphorhynchus bulbocolli. This parasite, with its long, slender proboscis and neck, perforates the mucosa, stratum compactum and tunica muscularis (Figure 11). Fibrous connective tissue surrounds the neck, neck bulb and proboscis (Figures 12-15) and this structure protrudes on the external surface of the intestine (Figure 16). It is large enough to be seen with the unaided eye.

In its attachment to the gut wall, P. bulbocolli destroys the normal structure of the intestine. The deleterious effect on the columnar epithelium is similar to that seen with infections of O. macilentis. The lamina propria, stratum compactum and muscularis layers are also destroyed at the site of attachment. The neck passes through these layers inside a tunnel that passes through the mass of fibrous connective tissue formed by the host (Figure 17). The connective tissue that surrounds the neck bulb and proboscis possesses two distinct layers and is referred to as a capsule. The inner portion is composed of several layers of epithelioid fibroblasts (Figure 15) which are absent

in nodules caused by O. macilentis. The outer layer is composed of dense interlacing strands of thick collagenous fibers with numerous fibroblasts and granular cells scattered among them. These observations confirm those of Chaircharn and Bullock (1967). They also noted masses of connective tissue in the intestinal wall that were not associated with living worms. This implies that the presoma of P. bulbocolli remains embedded after the death of the worm with the trunk being eliminated from the intestine. In a complete host-parasite system, tissue repair has been described by Bullock (1963). The damaged attachment area is initially invaded by fibroblasts, followed by collagenous fibers. The end result is that the site of former infection becomes filled with scar tissue.

Semotilus atromaculatus (Mitchill),

the Creek Chub

The intestine of creek chubs differs in several respects from that of the common sucker. The epithelial layer has a higher population of goblet cells, and the villi are much deeper and the folding is more intense (e.g., more folds per unit of distance). The layer beneath the lamina propria is not a true stratum compactum and is better called a submucosa. It is composed of parallel, thick, wavy, collagenous fibers. The tunica muscularis is thicker than in suckers, with a much more extensive circular muscle layer (Figure 18).

The intestinal mucosa at the site of attachment of Neoechinorhynchus saginatus shows the same denudation of epithelium with compression of the epithelium adjacent to the worm's trunk as occurs in suckers. The deepest penetration was to, but not through, the submucosa with destruction

of the lamina propria at the site of attachment. Occasionally, attachment occurred on the side of a villus. Only a short proboscis is morphologically adapted to attach to such a thin structure (there is not enough tissue present for a long proboscis to penetrate effectively). This type attachment only destroys the epithelium at the site of attachment and produces a very slight build up of connective tissue in the lamina propria (Figure 19).

When worms attached at the bases of villi, penetration was no deeper than the lamina propria. In these sites there is some build up of connective tissue in the lamina propria directly beneath the attached proboscis. Variations in the connective tissue reaction of the host may be due to the length of time the worm has been attached and the female/male differences mentioned previously. In most cases, there was no effect on the tunica muscularis, but occasionally a very slight thickening of the circular muscle layer directly below the site of attachment (Figure 20). There was no indication of any proliferation of collagenous fibers in the tunica muscularis.

In these attachments with limited host tissue reaction, there is little cellular response other than a slight increase in fibroblasts. In some instances, there is a greater influx of fibroblasts and granular cells, but the cellular response is always more pronounced adjacent to the site of proboscis attachment (Figure 21). It appears as if the lamina propria thickens beneath this site, preventing further damage to the intestinal wall.

Stizostedion vitreum (Mitchill),

the Walleye

The intestine of walleye differs from either of the two previously discussed fishes. The most significant difference is the presence of a well developed plicae circularis, branching villi and very thick tunica muscularis (Figure 22). It is not possible to accurately divide the connective tissue between the epithelium and the tunica muscularis into lamina propria and submucosa layers. However, there are bundles of smooth muscle randomly scattered in this connective tissue (Figure 23).

The attachment of Neoechinorhynchus cylindratus never extends beyond the lamina propria. The columnar epithelium sustains the same damage as described for other fishes at the site of worm attachment. Most often the hooks are embedded in, or slightly below, the dense connective tissue occurring directly beneath the epithelium (Figure 24). There is an abundance of fibroblasts and a proliferation of collagenous fibers directly beyond the point of attachment, leading to a thickening of the lamina propria/submucosal layer (Figure 25). There is also evidence of a limited infiltration of granular cells.

Larger worms (of both sexes) were generally embedded near the bases of villi (Figure 26). Smaller worms may be attached to the sides of villi (Figure 27). In the walleye, the attachment of N. cylindratus causes very little disruption of the lamina propria/submucosa, and no adverse effect in the tunica muscularis.

DISCUSSION

Certain general characteristics of acanthocephalan infections were observed in each of these host/parasite associations. In all fish observed, the presence of acanthocephalans apparently caused only mild effects. There was no obvious detriment to the general health of the fish. The histopathological effects were those of physical damage to the host's intestine, and the resultant fibrous and cellular host response. The amount of damage appeared to depend upon the depth of penetration of the proboscis. A complete destruction of the columnar epithelium at the site of attachment occurred in all infections. There also was a compression of the epithelium adjacent to the trunk of the worm. In addition, the lamina propria was disrupted to some extent in all infections. Further damage and the degree of host reaction was related to the depth of penetration.

A comparison of how related acanthocephalans affect different fish can be made by analyzing the infections caused by three members of the family Neoechinorhynchidae (e.g., Octospinifer macilentis, Neoechinorhynchus saginatus and N. cylindratus). All three worms have short, globular proboscises that are generally broader than long. Comparative measurements from the literature and from this study are presented below.

TABLE 1

AVERAGE SIZES OF ACANTHOCEPHALAN PROBOSCISES

Parasite	Proboscis length x width (μ M)	
	In Literature	This Study
<u>Octospinifer macilentis</u>	106 x 120*	78 x 101
<u>Neoechinorhynchus saginatus</u>	112 x 106*	91 x 96
<u>Neoechinorhynchus cylindratus</u>	150 x 172**	125 x 140
<u>Pomphorhynchus bulbocolli</u>	550 x 450** 3.30 x 1.15***	562 x 249 3.52 x 1.10

*Van Cleave and Bangham (1949)

**Van Cleave and Mueller (1934)

***Measurements for presoma in mm (width is at the neck bulb)

Though there is little or no sex differences in the dimensions of the proboscises, there is a general tendency for the females to embed deeper into the gut wall than do the males. However, in each species the females are about two times larger than the males. It would appear that the larger size of female worms requires a firmer attachment in order to maintain their position in the host's intestine.

Female O. macilentis produce a pronounced connective tissue and cellular response in the sucker. The response to N. saginatus in the chub and to N. cylindratus in the walleye produces a minimal hyperplasia and cellular response in the lamina propria. Since the proboscises of these acanthocephalans are so similar, it seems that differences in penetration and host response can be attributed to differences in the host intestines. For example, the sucker has fewer and lower mucosal folds as compared to the thick, tall folds found in the chub and walleye. Therefore, in the sucker, more of the worm is exposed to the peristaltic

movements of food than in fishes where deep, thick villi afford better protection for attached worms. Consequently, acanthocephalans occurring in suckers need a deep and firm attachment.

The sucker gut wall is much thinner than in that of either chubs or walleyes. A proboscis attaching to a thin intestine would be expected to cause more damage and to elicit a greater tissue reaction. The depth and degree of mucosal folding, and the thickness of the tunica muscularis in creek chubs is intermediate to suckers and walleyes. However, its gut morphology is closer to that of the walleye. As might be expected, the depth of attachment and the degree of host tissue response was intermediate, but resembled the minimal response seen in walleyes. The very deep, branched villi of the walleye provides excellent protection for acanthocephalans. Attachment to the lateral surfaces of the villi apparently can be maintained successfully. When attachment does occur at the base between villi, the thick lamina propria is the only layer, other than the epithelium, to be affected by the worm's presence.

Three conclusions can be made from the above discussion: 1) the depth of attachment by the proboscis of a neoechinorhynchid worm is primarily responsible for the variations in host reactions to its presence; 2) the depth and degree of mucosal folding determines where and how deeply the worm must attach in order to firmly anchor itself; 3) the relative thickness of the lamina propria and tunica muscularis seems to influence the amount of damage that attachment causes. Hypothetically, an extremely heavy acanthocephalan infection would probably produce less tissue problems in the thick walled intestine of walleyes than in the thin walled sucker gut. Creek chub intestine should show an intermediate tissue response.

Comparative responses of the sucker intestine to O. macilentis and P. bulbocolli allowed an analysis of how the presomas of these worms produce different host reactions. The proboscis of O. macilentis attached to a depth of $1/4$ - $3/4$ the thickness of the gut wall. The presoma of P. bulbocolli traversed the entire structure, with the tip of the proboscis situated just beneath the serosal covering. The latter association produced a far greater tissue and cellular infiltration. This is due in part to the unusual nature of the presoma of P. bulbocolli.

The inflated neck bulb increased the amount of connective tissue needed to wall off the presoma protruding on the outer surface of the gut wall. Thus, in common suckers, both the depth of penetration and variation in the structure of the presoma influence the intestinal damage and the host's tissue response. It would be interesting to observe the effects of long presomas in the intestine of walleyes. It could give insight as to how that fish reacts to deep penetrating acanthocephalans.

It appears that fish in this study are well adapted to the presence of acanthocephalans. Outwardly, they appear to suffer no ill effects from this type of parasitism. Their tolerance may be due to the physical structure of the intestine. This was especially apparent in the thick walled guts of walleye and creek chubs. In the common sucker, the extensive connective tissue proliferation was equally effective in protecting the fish from the worm's presoma. In no case was there any indication of bacterial infection.

Lastly, collection of N. saginatus in creek chubs taken from Pipestone County, Minnesota, is the first record of this parasite in the state.

APPENDIX

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APPENDIX I

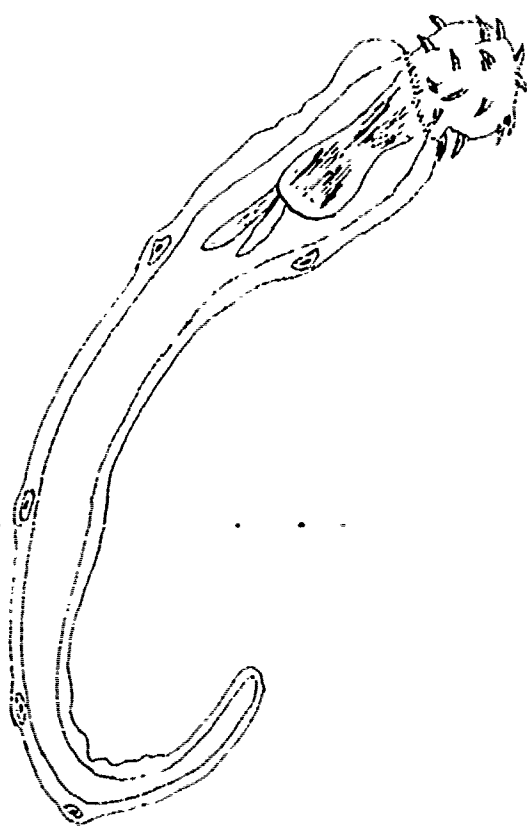
FIGURES 1-27

Fig. 1.--Octospinifer macilentis showing a proboscis bearing three rows of hooks with eight hooks per row.

Fig. 2.--Proboscis of Neoechinorhynchus saginatus showing three rows of hooks with six hooks per row.

Fig. 3.--Proboscis of Neoechinorhynchus cylindratus. Note similarity to that of Fig. 2.

Fig. 4.--Presoma of Pomphorhynchus bulbocolli showing a long thin neck, proboscis and its inflatable neck bulb.



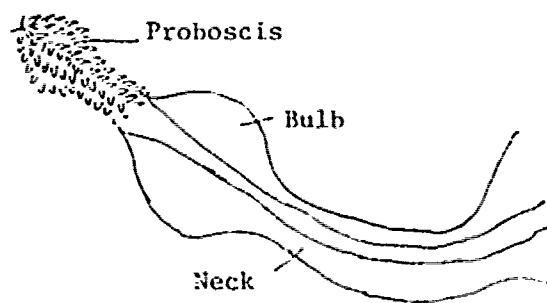
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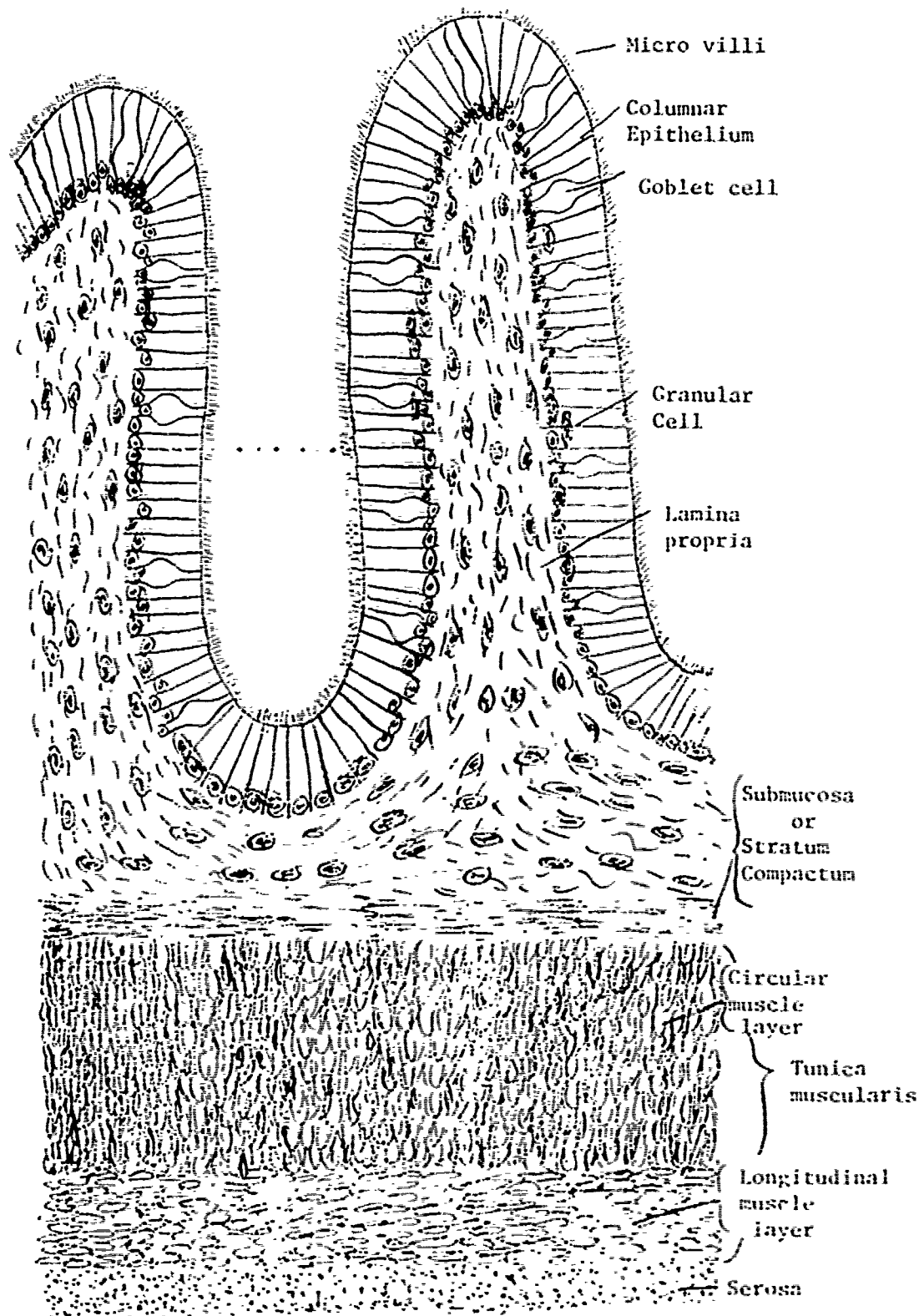


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Fig. 5.--Generalized sketch of fish intestine showing typical tissue layers and cells.



(5)

Fig. 6.--Normal intestine of Catostomus commersoni. Note the low simple villi, stratum compactum and wandering leukocytes. 395X

- a. Stratum compactum
- b. Wandering leukocytes
- c. Lamina propria

Fig. 7.--Longitudinal view of female Octospinifer macilentis that appears to have perforated the tunica muscularis of Catostomus commersoni. 95X

- a. Tunica muscularis
- b. Connective tissue nodule

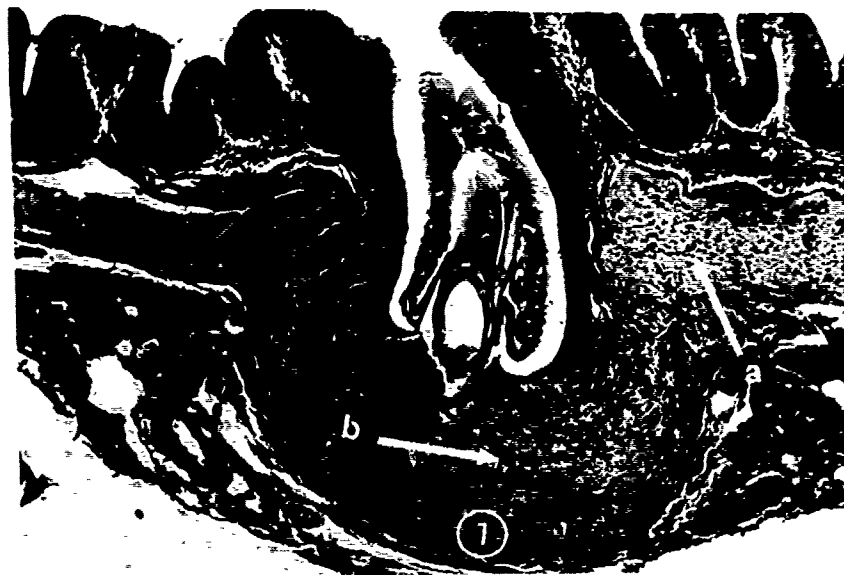
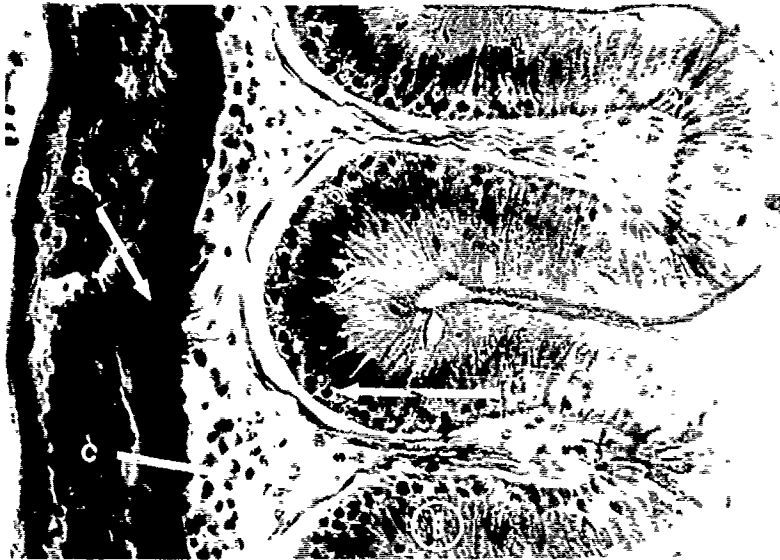


Fig. 8.--Longitudinal view of female Octospinifer macilentis in intestine of Catostomus commersoni showing destruction of the epithelium at the site of attachment with compression of the epithelium adjacent to the trunk of the attached worm. 80X

a. Compressed epithelium

b. Stratum compactum

Fig. 9.--Cross section view of the proboscis of a female Octospinifer macilentis embedded in a connective tissue nodule. 95X

a. Proboscis

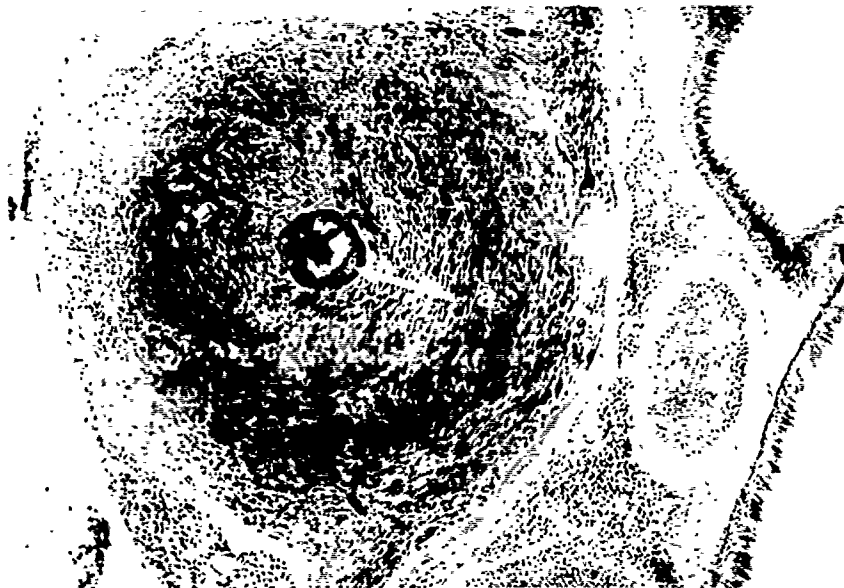
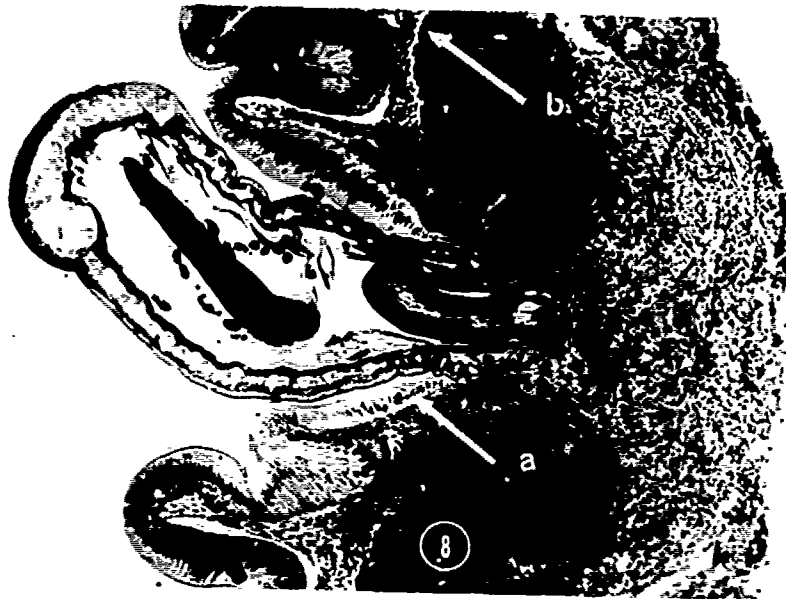


Fig. 10.--Longitudinal section of male Octospinifer macilentis in Catostomus commersoni. The proboscis reaches but does not pass through the stratum compactum. The host reaction shows granular cells near the proboscis in the lamina propria and some cellular infiltration and connective tissue build up in the muscularis, but no nodule formation. 130X

a. Stratum compactum

b. Lamina propria

Fig. 11.--Oblique section of Pomphorhynchus bulbocolli in Catostomus commersoni showing the trunk and the proximal portion of the neck where it penetrates the mucosa, stratum compactum and tunica muscularis. 85X

a. Stratum compactum

b. Tunica muscularis



Fig. 12.--Longitudinal section of the neck of Pomphorhynchus bulbocolli as it passes through the gut wall of Catostomus commersoni and the mass of connective that has built up on the exterior surface of the intestine. 70X

a. Tunica muscularis

Fig. 13.--Longitudinal section of the neck and neck bulb of Pomphorhynchus bulbocolli in Catostomus commersoni. 50X

a. Tunica muscularis

b. Neck

c. Neck bulb

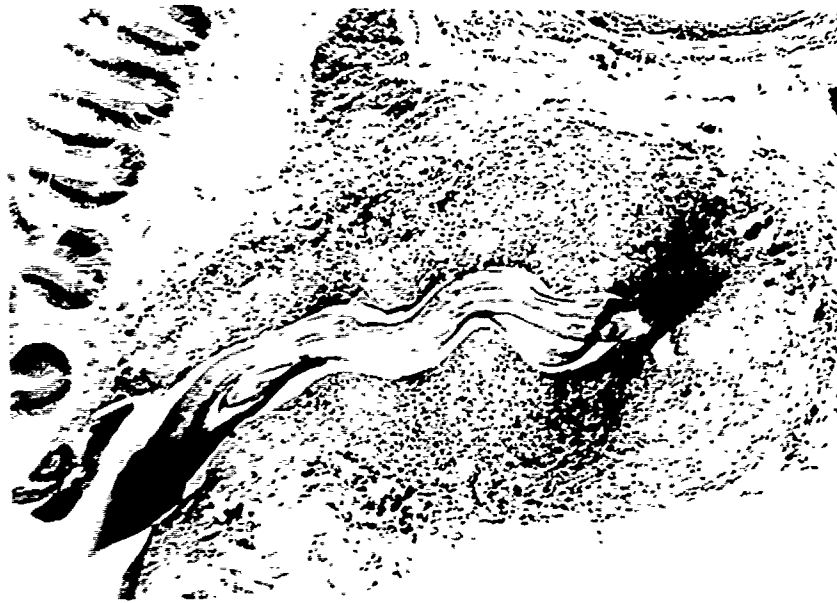


Fig. 14.--Cross section of the neck bulb of Pomphorhynchus bulbocolli. Note the thin neck of the worm as it passes through the bulb. 80X

a. Neck

Fig. 15.--Cross section view of the connective tissue capsule of Catostomus commersoni surrounding the proboscis of Pomphorhynchus bulbocolli. 170X

a. Tunica muscularis

b. Proboscis

c. Epithelioid fibroblasts

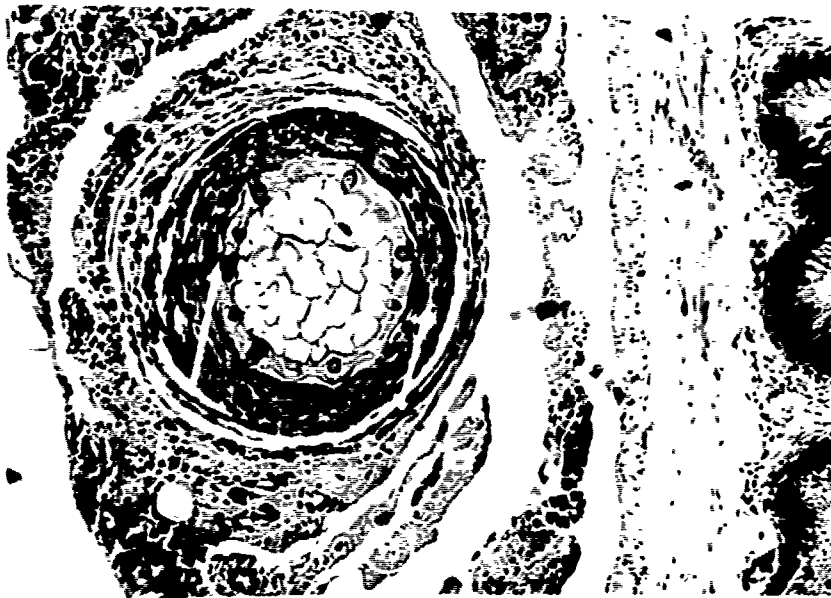
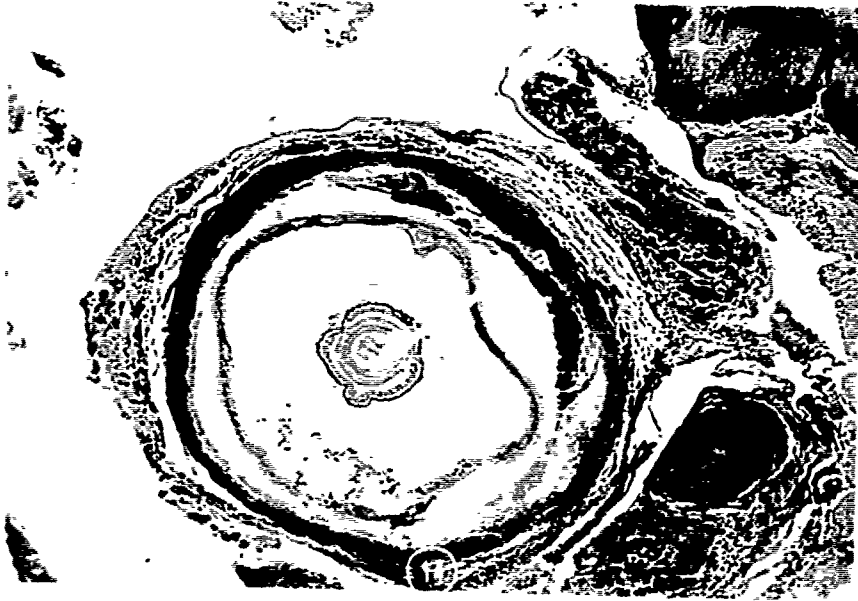
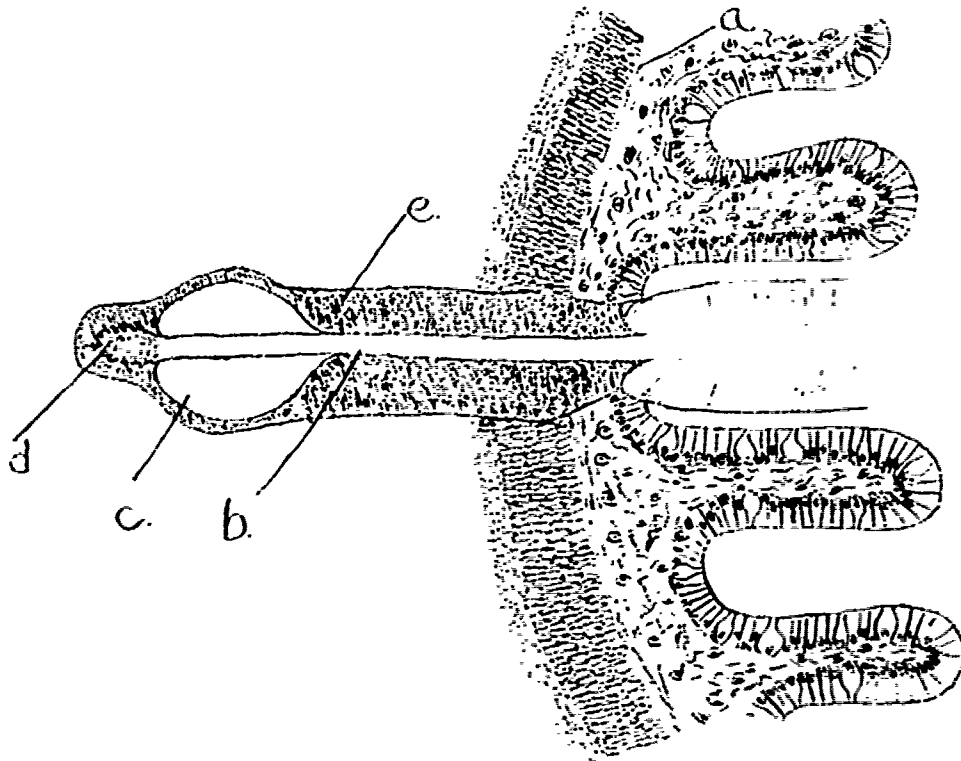


Fig. 16.--Diagrammatic view of the build up of connective tissue on the serosal surface of the intestine of Catostomus commersoni caused by Pomphorhynchus bulbocolli.

- a. Stratum compactum
- b. Neck
- c. Neck bulb
- d. Proboscis
- e. Connective tissue

Fig. 17.--Cross section view of a connective tissue "tunnel" formed by Catostomus commersoni in response to the neck of Pomphorhynchus bulbocolli. 65X

- a. Tunica muscularis
- b. Neck



(16.)

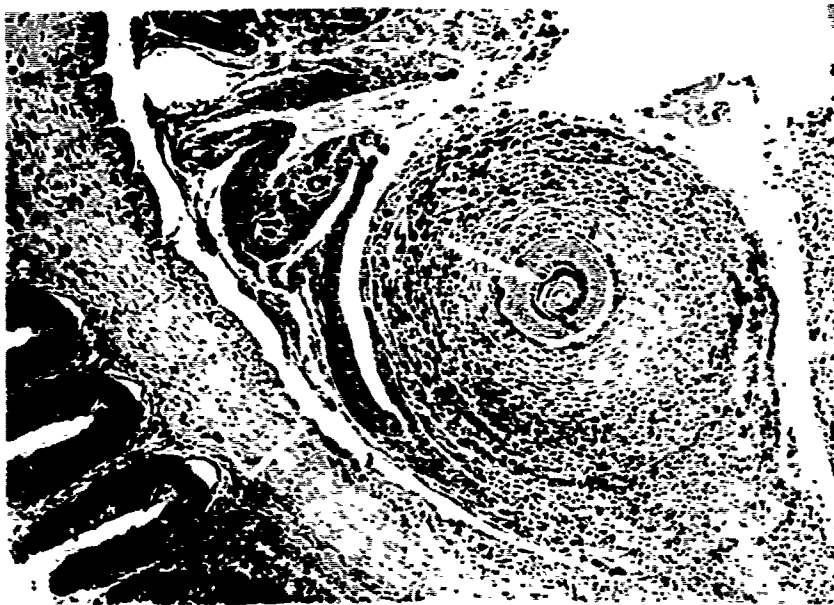


Fig. 18.--Section showing normal intestine of the creek chub, Semotilus atromaculatus. Note the abundance of goblet cells and the deep villi. 60X

a. Tunica muscularis

Fig. 19.--Attachment of a Neoechinorhynchus saginatus to the side of a villus. Note the physical destruction of the epithelium, the disruption of the lamina propria, and the very slight tissue reaction to the worm. 145X

a. Proboscis



Fig. 20.--Longitudinal section of Neoechinorhynchus saginatus attached at the base of the villi in Semotilus atromaculatus. Note an increase of connective tissue beneath the attached proboscis in the lamina propria. 65X

a. Lamina propria

Fig. 21.--Neoechinorhynchus saginatus showing the increased cellular response in the lamina propria near the attached proboscis in the intestine of Semotilus atromaculatus. 90X

a. Lamina propria



Fig. 22.--Normal intestine of Stizostedion vitreum showing the plicae circularis, branched villi, and the very thick tunica muscularis. 40X

a. Tunica muscularis

Fig. 23.--Stizostedion vitreum intestine with the randomly scattered bundles of smooth muscle (arrows) within the lamina propria.

a. Dense connective tissue

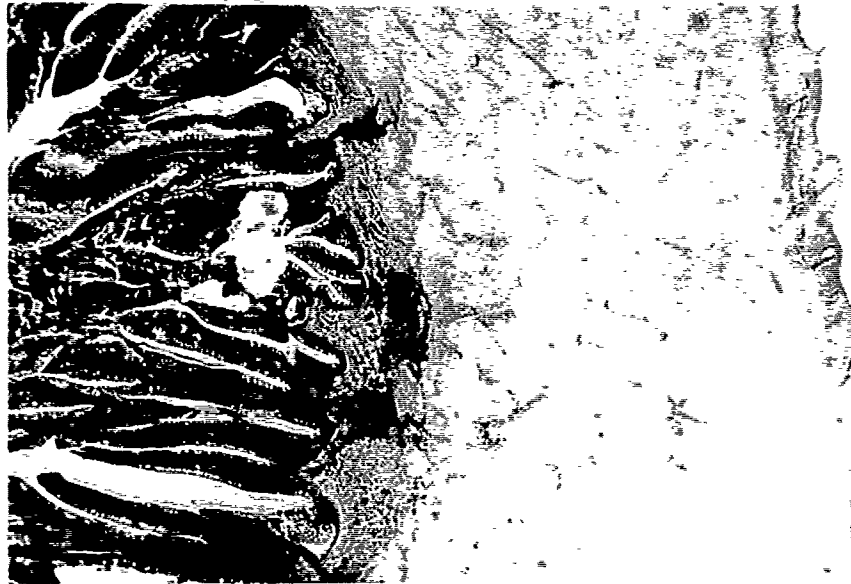


Fig. 24.--Longitudinal section of Neoechinorhynchus cylindratus in the intestine of Stizostedion vitreum. Note the anchoring of hooks in the dense connective tissue directly beneath the columnar epithelium. 375X

a. Hook

Fig. 25.--Longitudinal section of Neoechinorhynchus cylindratus in the intestine of Stizostedion vitreum. Note the cellular infiltration and increased connective tissue beyond the attachment site. 185X



Fig. 26.--Longitudinal section of Neoechinorhynchus cylindratus showing attachment at the base of the villi. Note the partially retracted proboscis and elevated host tissue. 105X

a. Proboscis

b. Elevated host tissue

Fig. 27.--Cross section of a Neoechinorhynchus cylindratus proboscis attached to a villus. Note the six hooks per row characteristic of the Neoechinorhynchidae. 200X



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